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Heavy Chain Only Antibodies: A New Paradigm in Personalized HER2+ Breast Cancer Therapy

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SUMMARY

Unlike conventional antibodies, heavy chain only antibodies derived from camel contain a single variable domain (VHH) and two constant domains (C_H2 and C_H3). Cloned and isolated VHHs possess unique properties that enable them to excel conventional therapeutic antibodies and their smaller antigen-binding fragments in cancer targeting and therapy. VHHs express low immunogenicity, are highly robust and easy to manufacture and have the ability to recognize hidden or uncommon epitopes. We highlight the utility of VHH in design of new molecular, multifunctional particulate and immune cell-based systems for combating HER2+ breast cancer.

Breast cancer is the second most common cancer that occurs in women. More than 1.1 million cases are diagnosed annually and of these more than 410,000 patients die from breast cancer worldwide.¹ Malignant tumors usually express high levels of plasma membrane specific antigens, where some of these contribute to or promote the cancer phenotype through signaling and are considered as important therapeutic targets.^{2,4} Aberrant expression or activity of two members of the human epidermal growth factor family of receptors (HER), HER1 and HER2, have been connected to 20–30% of breast cancer cases.⁵ HER2 is a signaling tyrosine kinase receptor that causes increased cell proliferation, tumor invasiveness, accelerated angiogenesis and reduced apoptosis, ultimately translating into an aggressive disease, resistant to traditional systemic therapy, increased probability for recurrent disease and decreased survival.^{6,7} To date, only two drugs for targeted therapies of HER2-overexpressing tumors have been approved for human use. These include Tykerb (lapatinib) that targets the intracellular domain of HER2 and Herceptin (trastuzumab) which targets an extracellular part of HER2.^{8,9} There are complications with these therapies as well as with combination approaches.^{7,10} These include primary or intrinsic resistance to lapatinib as well as resistance and particularly cardiotoxicity with trastuzumab.¹⁰ Recent

attempts have further exploited the endocytic properties of HER2 by tagging HER2 monoclonal antibodies to drug carriers such as liposomes.¹¹ This approach not only creates multivalent antibody constructs capable of modulating malignant cell survival signaling pathways via extensive cross-linking of target/antibody complexes, but further allows for the delivery of chemotherapeutics and killer genes to HER2 expressing cells.¹¹ Although promising results have been obtained, there are important nanoengineering problems involving construct design. These include complications with generation of sufficient functional surface antibody density without triggering immune responses (an important safety issue), antibody orientation and reduced antibody functionality (arising from chemical conjugation procedures). These concerns are not easily resolved even with the use of conventional antibody domains which lack the Fc region such as Fabs, diabodies, single chain variable fragments, bispecific antibodies and variable domains, especially when the antibody molecule possesses therapeutic activity.¹¹⁻¹³

The discovery of heavy chain antibodies has given rise to unprecedented opportunities in impacting cancer therapy.¹⁴ These unique form of camelid-derived antibodies lack the entire light chain and the C_H1 domain and are only composed of a single variable domain termed VHH (Fig. 1).

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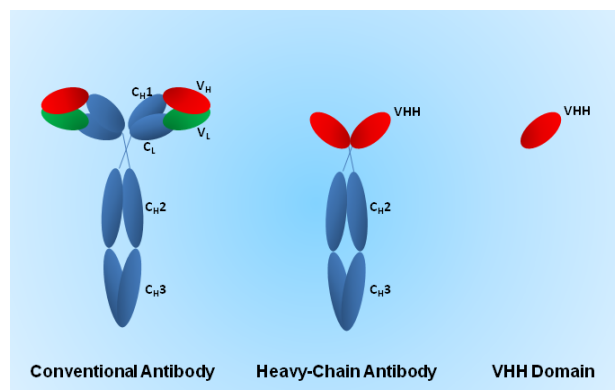


Fig. 1. Schematic representation of conventional and heavy-chain antibodies. Unlike conventional antibodies, heavy-chain antibodies contain a single variable domain (VHH) and two constant domains (C_{H2} and C_{H3}). Cloned and isolated VHH domain is highly stable and exhibit full antigen-binding capacity of the original heavy-chain antibody. There are ongoing efforts on phage display formats, library design and selection technologies for isolation of VHH domains against a diverse set of target antigens.¹⁵

Recombinant VHHs are small (15-20 kDa) and strictly monomeric; they bind their target with nM affinity as well as with being stable in a broad pH and temperature ranges.¹⁶ Molecular manipulation is also easier with VHH; this facilitates the production of multivalent formats of monoclonal antibodies compared with conventional recombinant antibodies and their fragments, which is problematic due to aggregation and reduced affinity (i.e., mispairing of variable H and L domains). Moreover, VHH often binds to epitopes that are less immunogenic for conventional antibodies.¹⁶ Another advantage is that they are well expressed in bacterial expression systems; hence, they are cheaper and easier than standard monoclonal antibodies to produce (at mg/L culture). Most importantly, heavy chain antibodies show 80% sequence homology to human VH fragments and therefore exhibit low immunogenicity.¹⁶ Collectively, these features make VHHs ideal small-sized functional candidates for (a) conjugation to macromolecular and nanoparticulate systems with optimized pharmacokinetic profiles, thus opening the path to new and sophisticated design solutions for targeted cancer therapy, and (b) T cell therapy which involves the adaptive transfer of tumor antigen specific T lymphocytes into cancer patients.¹⁷⁻²¹

Recently, we provided the proof of concept that combination of a cancer specific VHH and gene therapeutics can exert dramatic effects on cancer cell

recognition and cancer cell-specific killing, while offering no harm to non-target cells.¹⁹ This was done using a family of VHHs with specificities for DF3/Mucin 1 (MUC1) antigen, which is an aberrantly glycosylated glycoprotein over-expressed in a number of tumors including breast cancer in conjunction with transcriptional targeting of truncated Bid killer transgene for directed killing of MUC1 over-expressing tumor cells. To tackle the proposed problems in HER2-positive breast cancer and encouraged by unprecedented success of general VHH approach in cancer cell destruction, we (unpublished observations) and others have generated a panel of anti-HER2 VHHs.²⁰ The availability of these VHH libraries offers a unique opportunity for paving the path towards molecular imaging and assessing intratumoural and intertumoural heterogeneity of HER2 expression, combination therapies as well as design of multifunctional HER2-targeted macromolecular and particulate therapeutic/theranostic systems. Furthermore, by matching epitope-distinct binding, VHH-based protocols may be personalized thus improving therapeutic efficacy and outcome. However, the safety of these approaches must also be considered in relation to innate immunity.²²⁻²⁶ Another interesting strategy is HER2-directed oligoclonal T cell therapy, since VHH, by virtue of its small size, can endow great targeting ability to chimeric antigen receptor-engineered T cells, while minimizing adverse effects. The validity of this approach is also based on our recent observation with target-specific T cells bearing chimeric constructed receptors whose antigen binding moiety was comprised of a panel of camelid VHHs recognizing tumor-associated glycoprotein-72 (a cell surface molecule with mucin-like characteristics and overexpressed on a range of tumors including breast and colorectal cancers).²¹ These genetically engineered T cells expanded after antigen encounter and exerted potent tumor-specific functions. These approaches have the potential to reverse multiple tumors immune evasion mechanisms and avoid chimeric antigen receptor immunogenicity. Work on construction of genetically engineered T cells, bearing chimeric constructed receptors with HER2-specific camelid single domain antibodies as targeting agents, is ongoing and being tested both *in vitro* and validated *in vivo* animal models.

Finally, recent advances in nanotechnology are expected to rapidly advance 'patient-centric' translational approach to cancer treatment.²⁷ Potential examples

include micro-fluidic and micropatterning fabrication approaches (Fig. 2) for immune cell confinement in two- or three-dimensional (hydrogel-based) scaffolds. This could potentially allow parallel individual processing, training and selection of millions of T cells and even dendritic cells, thus opening new avenues for efficient planning and implementation of personalized immune-cell therapies for cancer in general.

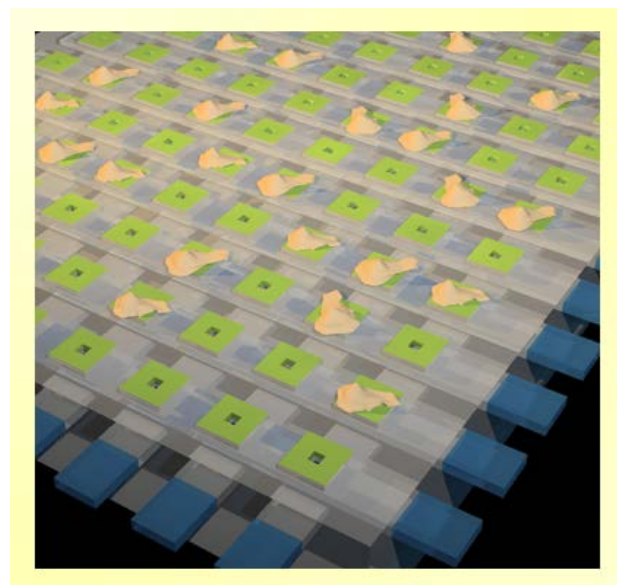


Fig. 2. Schematic representation of a two-dimensional cell-specific harvesting micro-fluidic platform. The device will allow processing of millions of cells through cell-specific immobilization at the micron-scale areas (square) covered with microcontacted printed 'anchor' ligands. Non-specific bound cells are removed by increasing the flow through the device. Attached cells may be modulated through appropriate micropatterning on conducting polymers and materials flow through the device, and cell responses may be monitored. Cell-specific harvesting may be achieved through the control of electrical signal.

Competing interests

The authors declare no competing financial interests.

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